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SOME OBSERVATIONS ON *CHAOBORUS PUNCTIPENNIS* SAY (DIPTERA, CULICIDAE)

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Aquatic biologists have long been familiar with the beautiful and interesting larvae of *Chaoborus* which are found in most of our lakes. Their peculiar habit of migrating daily from the bottom of the lake to the surface and back again is well known. The data reported here are concerned not so much with this interesting habit but rather with the general ecology of the species.

During the summers of 1937 and 1938 the author was engaged in studies of bottom productivity of lakes, part of the programme of the Ontario Fisheries Research Laboratory. Headquarters of the Laboratory were then, as now, in Algonquin Park, Ontario. The lake on which this particular study was made is Costello lake, a brown-water lake 90 acres in extent and with a maximum depth of twenty metres. Costello is typical of the many small Algonquin Park lakes.

The standard method of investigating bottom productivity is to dredge up small areas of the lake bottom and carefully count all the animals found. Such dredgings are taken at different depths in the lake and an estimate of the total numbers of animals present is made. The objection to this technique is that it fails to give information as to the rate at which bottom dwelling insects are emerging as adults. The actual productivity of bottom animals cannot, therefore, be calculated.

To overcome this objection the tent-trap method of collecting adults was used. In this method, small floating tents were anchored on the surface of the lake so that insects emerging beneath them would be captured. These tent-traps were lifted and the insects removed each day. As well as this tent-trap method, the regular dredging technique was employed, so that data on both larvae and adults of aquatic insects were secured. The Chironomidae formed the greatest part of the catch; data on this group are being published separately (Publications of the Ontario Fisheries Research Laboratory). *Chaoborus punctipennis* was also prominent, both in the dredgings and the tent-traps.

The larvae of *C. punctipennis* were found only below the thermocline, i. e., in water of seven to eighteen metres depth. Over this range their distribution was quite uniform, probably as a result of their migratory habits. Larvae were taken in the dredge early in the spring of both summers (1937 and 1938). They were from seven to eight millimetres long and had evidently just changed from the free swimming to the bottom dwelling mode of life (Eggleton, 1932). Dredgings throughout May revealed increasing numbers of larvae on the bottom (up to thirty to forty per square foot). In early June there was a rapid falling off in numbers, and many of the larvae taken were pupating. Coinciding with this decrease in numbers of larvae, adults began appearing in the tent-traps. It is interesting in this connection that the emergence occurred at the surface of water of all depths; the very active pupae may evidently stray or be carried far from their larval habitat. During the latter half of June and the first half of July only one or two larvae (and these were pupating) were found in the dredgings. Adult emergence was vigorous over this period. This suggests

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that the pupae must be entirely free swimming as few were found in the dredgings. The peak of adult emergence occurred during the last week of June and the first week of July (figure 1).

Around the middle of July small larvae, similar to those found in the spring, began to appear in the dredgings (coincident with the cessation of emergence). Numbers and size of the larvae rapidly increased to a maximum about the middle of August. At this time the larvae began to decrease in numbers and pupating larvae were found. Soon after this, emergence began again

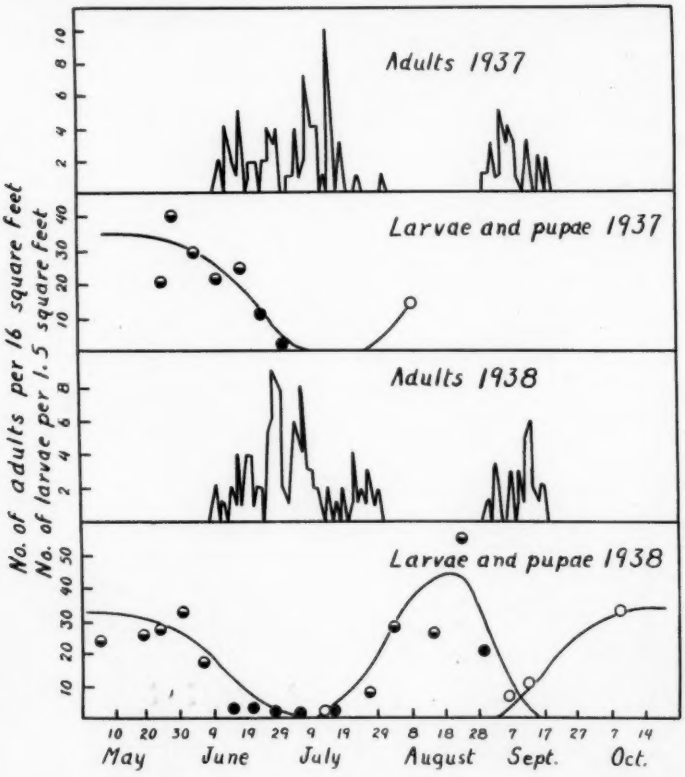
Adults 1937
Larvae and pupae 1937
Adults 1938
Larvae and pupae 1938
No. of adults per 16 square feet
No. of larvae per 1.5 square feet
2 4 6 8 10
10 20 30 40
2 4 6 8
10 20 30 40 50
10 20 30 9 19 29 9 19 29 8 18 28 7 17 27 7 14
May June July August Sept. Oct.

Figure 1. The variations in larval abundance and the emergence of *Chaoborus punctipennis* in Costello lake. Open circles indicate young larvae (under seven millimetres); half black circles indicate mature larvae (over seven millimetres); all black circles indicate pupating larvae and, in a few cases, pupae. (Larval data are incomplete for 1937 when collecting was discontinued in August.)

(August 29, 1938). This emergence continued to about the end of September after which no adults appeared until the following year. Small larvae were again found upon the bottom about September 15; these were the ones which emerged in June of the following year (figure 1). Larvae collected November 20, 1938, and kept in an aquarium at 15°C. began to emerge on January 17, 1939.

These data reveal several interesting points. Most striking is the remarkable correspondence between adult emergences in the the two years. Emergence lasted over the same period each summer for both the first and second generations. The number of adults that emerged is also the same for both years. This

close adherence to schedule is probably because of the constancy of the larval environment, which, being deep water, is well insulated from minor climatic variations.

That there is a high mortality of pupae is shown by the data. Many more larvae were found on the bottom than adults were captured in the tent-traps (per unit area). In 1938 an average of 297 larvae per sixteen square feet was found on the bottom before the June-July emergence. The total number of adults which emerged from this area was 121; 59 per cent of the larvae failed to emerge. It seems probable that the free swimming pupae are an easy prey for fish, and indeed lake trout have been caught whose stomachs were well filled with *Chaoborus* pupae.

Chaoborus punctipennis in Costello lake lives in deep water varying from 4° to 8°C. In such a cold habitat it is truly remarkable that the species is able to run through two generations in one summer. Certain shallow water chironomids in Costello lake living in a habitat varying in the summer from 15° to 25°C. have two generations each year, but the deep water chironomids, in the same habitat as *Chaoborus,* often require two years for one generation. The migration each night to the warmer surface waters of the lake is the only explanation which suggests itself. The hours of darkness spent in warm water apparently serve to speed up the growth rate of the larvae of *Chaoborus punctipennis,* enabling them to far outstrip the non-migrating chironomids.

LITERATURE CITED

Eggleton, F. E. 1932. Limnetic distribution and migration of *Corethra* larvae in two Michigan lakes. Papers of the Michigan Academy of Sciences, Arts and Letters, 15.

THE FEEDING HABITS OF CERTAIN LEAFHOPPERS*

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The feeding habits of certain leafhoppers (Cicadellidae) are of particular importance, not only because of the diverse nature of the injury produced by different species, but primarily because a number of species are vectors of some very destructive plant viruses. An investigation of the feeding habits of certain species was undertaken to learn the nature of the injury within the host plant, especially the particular tissues fed upon and their relation to the external symptoms, and to bring out any features of the methods of feeding which might bear some relation to the transmission of viruses. Primary attention was given to the plum leafhopper, *Macropsis trimaculata* (Fitch), because of its role as the vector of the viruses of peach yellows and little peach. *Macropsis insignis* (Van Duzee) was included because of its availability and close relationship to *M. trimaculata* and the possibility that it may also be involved in the transmission of peach viruses. The white apple leafhopper, *Typhlocyba pomaria* McAtee, was selected as an example of a large group of species which produce white mottling of foliage. The potato leafhopper, *Empoasca fabae* (Harris), the feeding habits of which have been studied by other workers, was included in the present investigation largely for comparison with the other species.

The scope of the investigation was limited to histological studies of injured tissues, and no attempt was made to deal with such secondary effects as those produced by *Empoasca fabae* which are properly problems of plant physiology.

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REVIEW OF LITERATURE

Horne and Lefroy (15) investigated the punctures of *Eupteryx atropunctata* Goeze and *Chlorita* (*Empoasca*) *viridula* Fall. on potato. In foliage injured by these species, which produce the white spotting characteristic of many leaf-feeding cicadellids, they found that the assimilatory tissue was destroyed and the white spots resulted from empty air spaces. *Eupteryx auratus* Liv. and *Zygima* (*Erythroneura*) *pallidifrons* Edw., which cause similar injury on potato were studied by K. M. Smith (25), who believed the white spots to be caused by destruction of the chlorophyll in the mesophyll cells by the action of the saliva, the cell walls remaining intact. The area of the spots increased somewhat after feeding had been completed and the overlying epidermis usually collapsed. Both the palisade layers and the vascular system were tapped, but no preference was shown for the neighbourhood of the veins. The passage of the stylets was intracellular, with a thin lining, the stylet sheath, staining pink with safranin.

Smith and Poos (24) investigated the feeding habits of six species of *Empoasca*. Five of these, *E. bifurcata* De L., *erigeron* De L., *filamenta* De L., *abrupta* De L., and *maligna* (Walsh), which produce white spotting similar to that of the foregoing species, fed chiefly in the palisade layers and to some extent, especially in the case of *maligna*, *erigeron*, and *bifurcata*, on the spongy mesophyll. While vascular tissue was penetrated in a few instances this occurrence appeared to be accidental and there was no evidence that the phloem was sought. Cells in the injured areas were torn open and emptied of their contents. Only those actually penetrated were injured; adjacent cells remained normal and there was no evidence of diffusion of a toxic saliva. The spots on mature leaves did not enlarge. The sheath material, staining bright red, was found in irregular accumulations in the middle lamella between cells of the cortex or the mesophyll, and only occasionally within or surrounding the cells. The sixth species, *Empoasca fabae* (Harris), studied by the same workers produces an entirely different type of injury. There is usually no visible injury at the immediate point of feeding, but various secondary symptoms including wilting, chlorosis, pigmentation, and frequently eventual necrosis, of parts of the leaf distal to the punctures appear later. This species was found to be primarily a phloem feeder, the cells of this tissue being punctured and torn or distorted, or filled, partially or completely, with the red-staining sheath material. The path of the stylets was intercellular through the parenchyma of the midrib or the cortex of stems, with deposition of sheath material along the track which did or did not form a definite tubular sheath according to the consistency of the tissues. In cases where wilting occurred it was shown that the xylem had been punctured and the vessels filled with sheath material.

King and Cook (18), working with *Homalodisca triquetra* (Fab.) and *Graphocephala versuta* (Say) which feed on stems of cotton, found that the punctures always ended in the phloem or occasionally in the xylem, the stylet track being lined with a sharply defined sheath.

In a study of the feeding habits of *Eutettix tenellus* (Baker), Bennet (2) found that the chief source of nourishment in beet foliage was in the phloem, the insects quickly dying when fed on tissues not containing the vascular elements. The punctures were relatively straight and largely intracellular and lined with the sheath material. Most of the parenchyma cells pierced remained alive and apparently normal for many days, the puncture being sealed off from the cell contents by the sheath material. Brandes (3) reported that *Draeculacephala mollipes* (Say) fed in the vascular bundles, but the very large size of the stylets made it difficult to determine the exact tissue preferred. No definite sheath was formed but a few traces of red-staining secretion were found along the stylet track. Storey (26) found that *Cicadulina mbila* (Naude) sought the phloem of corn leaves and deposited a well-formed tubular sheath.

The feeding habits of other families of Homoptera are of interest because of the light which they throw on the development of feeding habits within the Cicadellidae. Most of the species in several different families which have been studied feed largely in the phloem. Davidson (4), Horsfall (16), Tate (27), Brandes (3), and others have found this to be true of many species of aphids; K. M. Smith (25) of *Trialeurodes* (*Asterochiton*) *vaporariorum* Westw. (Aleurodidae) and *Pseudococcus* (*Dactylopius*) *longispinus* Targ.-Tozz. (Coccidae); King and Cook (18) of *Stictocephala festina* (Say) (Membracidae); and Withycombe (30) of *Monecphora* (*Tomaspis*) *saccharina* Dist. (Ceropidae). Among the few exceptions, the punctures of *Aspidiotus hederae* Vallot (Coccidae) investigated by K. M. Smith (25) ended in the cortex of *Cordyline*. In the Chermidae, the gall-forming *Pachypsylla* spp. studied by Wells (29) feeds in parenchymatous tissue, and *Paratrioza cockerelli* Sulc. was found by Eyer and Crawford (8) to feed largely in the bundle sheaths. Pussard (22) mentions briefly that the punctures of several species of chermids penetrate to the cambium and frequently beyond to the xylem elements.

The Sheath Material. The stylet sheath or sheath material referred to in the previous section has been found by many workers in the punctures of most groups of the Homoptera and some of the Heteroptera. A review of its occurrence and speculation on its nature and origin was published by F. F. Smith (23). The same author proved by exhaustive microchemical tests that the sheath material of *Empoasca fabae* and the membarcid *Stictocephala festina* was at least largely of animal origin and consisted mostly of protein with possibly some pectic substances. Some of the tests produced reactions similar to those given by chitin.

Bennet, in his study of *Eutettix tenellus*, was able to follow the process of sheath formation by allowing the insects to feed in liquid through a membrane; a secretion exuded at the tip of the stylet fascicle coagulated to form a hyaline sheath about the stylets. Storey (26), using a similar technique, observed that the sheath of *Cicadulina mbila* was formed in the same manner.

METHODS OF STUDY

Nymphs and adults of *Macropsis insignis* and adults of *M. trimaculata*, collected from wild plum (*Prunus americana*) at various points in the Niagara Peninsula, were allowed to feed on potted peach and Myrobalan plum seedlings in a screened insectary; a few were also placed on peach trees in the orchard. The hoppers were confined individually or in pairs on a short length of twig by means of a piece of glass tubing slipped over the twig, the ends being plugged with cotton. Identification was checked by comparison with series of the two species determined by Mr. G. S. Walley, Division of Entomology, Dominion Department of Agriculture.

All stages of *Typhlocyba pomaria* were collected from apple at Vineland Station and confined on the leaves of potted apple trees or on apple foliage in the orchard by means of celluloid micro-cages. A considerable amount of naturally injured foliage was also examined.

Empoasca fabae was collected from a row of heavily infested broad beans and later from potatoes at Vineland Station. Male genitalia of several examples were compared with those of specimens determined by Dr. R. H. Beamer. The insects were confined on potted apple trees in the same type of micro-cage used for *T. pomaria*, or in some cases allowed to feed on exposed leaves. Injured foliage was also taken from orchard trees.

Some material, chiefly of *T. pomaria*, was sectioned free-hand and examined in water or lactophenol to follow the early development of injury, but the bulk of the material was fixed and preserved in formol-acetic-alchohol or 70

per cent alcohol. Dioxan was used as a dehydrating agent before embedding and gave very satisfactory results, but the mixtures of dioxan, xylol, and wa: recommended by McWhorter and Weier (20) gave much better and faster infiltration than dioxan alone. Leaves were sectioned at 10 microns and most of the twigs at 12 microns, with some of the latter at 5 microns for study of histological detail. The sections were stained in Delafield's haematoxylin and safranin, or safranin and light green; the latter combination, with the light green in clove oil, gave much clearer differentiation of the punctures and was used for most of the material.

Typhlocyba pomaria McAtee

Feeding by *Typhlocyba pomaria* produces very conspicuous external symptoms in the form of small whitish spots on the foliage, in this respect being typical of the majority of American species within the subfamily Typhlocybinae (Eupteryginae). The author has found this type of injury produced by *Typhlocyba* (16 species), *Erythroneura* (at least 14 species), *Empoasca* (4 species in addition to those studied by Smith and Poos), *Dikraneura* (*mali* Prov. and *cruentata* Gill.), *Alebra albostriella* (Fall.), *Forcipata loca* De L. and Cald. and *Eupteryx melissae* Curt., the only exceptions observed or found in the literature being *Empoasca fabae* and possibly a few additional species in the same genus.

Under low magnification the individual spots have a fine stippled appearance; typically they are more or less circular but somewhat irregular and diffuse in outline, and very frequently have a small central spot of apparently normal green tissue. Frequently they appear as a number of more or less separate smaller spots surrounding the central area, or as an incomplete ring. Incomplete feeding areas of this type were especially frequent when the hoppers were crowded in a small cage where they disturbed one another while feeding. When foliage is very severely injured the spots become confluent and the individual feeding areas are no longer discernable. The visible injury produced by *T. pomaria* is very similar to that described by Smith and Poos for *Empoasca bifurcata, erigeron, filamenta, abrupta,* and *maligna.*

T. pomaria in all stages feeds exclusively on leaves which are fully expanded, and does not normally occur on young leaves of growing shoots.

Sections of injured leaves showed that each spot was the site of a single feeding puncture, the mouth stylets being forced through the lower epidermis and the cells of the palisade layers destroyed for some distance about the entrance. The puncture through the lower epidermis was comparatively inconspicuous and, except for the presence of the brightly stained sheath material to be described later, would have been difficult to detect. The stylets were usually thrust directly through an epidermal cell, but in many cases they entered beside a stoma between a guard cell and the adjacent epidermal cell or, in roughly 10 per cent of the punctures, through the stoma itself.

The stylets passed through the large intercellar spaces of the spongy parenchyma to reach the palisade layers above. An occasional cell of the spongy layer was pierced, but the cells are so few and scattered that the insect would obtain little nourishment in this loose tissue. By far the greater part of the feeding was done in the palisade layers, the cells being punctured and emptied of their contents over the area showing external symptoms. Uninjured cells, singly or frequently in groups, were left scattered among the empty ones, especially about the margin of the feeding area, and at the centre was usually a similar but larger group which was visible externally as the characteristic central green spot. Frequently only one or two of the two or three palisade layers normally present were injured over part of the feeding area.

In sections of leaves fed upon by nymphs of the earlier instars there appeared to be a distinct tendency for the injured areas to be located above or about the very small vascular strands, although they were by no means invariably so placed. The vascular tissue, however, was rarely attacked even when the parenchyma above was destroyed. Occasionally some of the cells of the bundle sheath were injured and in a few cases the stylets had penetrated the xylem or phloem, but the scarcity of this type of injury seemed to indicate that the vascular strands were punctured more or less accidentally and that the hoppers made no effort to locate this tissue. A few cells of the upper epidermis were pierced but the latter generally remained intact over the injured areas.

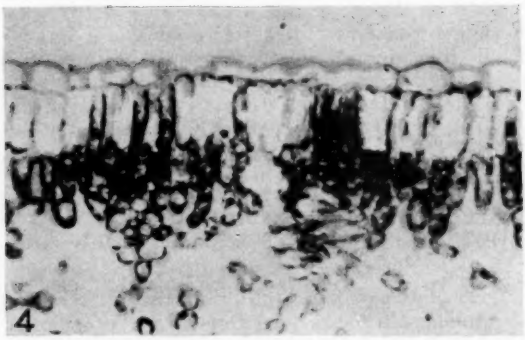
The internal injury produced by *T. pomaria* was essentially the same as that caused by the mesophyll-feeding species of *Empoasca* studied by Smith and Poos, although the latter species appeared to feed to a greater extent on the spongy mesophyll, probably because of the denser texture of this tissue in alfalfa leaves.

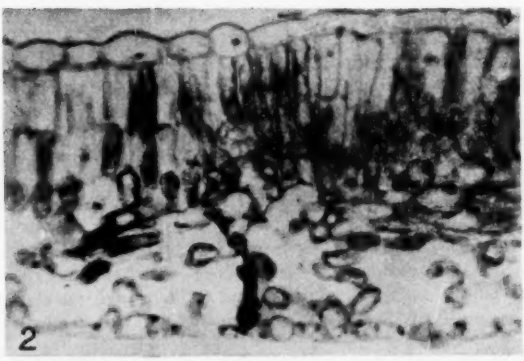
The contents of the palisade cells were usually removed completely, but scattered among these cells were some in which a portion of the contents remained as a firm homogeneous mass dried up in the ends of the cell, pale amber-coloured or a deeper brown in free-hand sections of fresh material or staining deeply with safranin in paraffin sections. Such partly emptied cells were particularly abundant when the leafhoppers fed under crowded conditions in cages, and in this case were presumably caused by the insects disturbing one another while feeding. The cell walls, although frequently torn to a considerable extent by the mouth parts of the insect, usually retained their normal shape and size after removal of their contents, and the injured areas did not collapse.

The process of removal of the cell contents was followed by taking leaves at different intervals after the hoppers had begun to feed and making freehand sections of the fresh material or fixing it for later paraffin sectioning. About five minutes after commencement of feeding the contents of injured cells had become broken down into a homogeneous mass in which the chloroplasts were not visible and the chlorophyll had become bleached to a very pale green. Fixed and stained sections of this stage showed the remains of the contents as diffuse, somewhat fibrous and lightly-staining material in which more deeply stained granules might have been starch grains. In leaves fixed an hour later no further changes were noticed, except that a greater number of cells had their contents completely removed, but 24 hours later what remained of the contents had condensed in the ends of the cells to form the brown masses previously mentioned. No further changes could be seen in injury at least four weeks old.

It was evident that some cytolyzing agent was introduced by the insect into the cells to produce a form of extra-intestinal digestion before the contents were ingested. Even if its action had not been observed it would be necessary to postulate some agent to cause the breakdown of the cell contents before the latter are removed, as the chloroplasts are too large to pass through the lumen of the stylet bundle. When sections made while the hopper was still feeding were treated with iodine many starch granules were seen lying free in the cytolyzed cells, the chloroplasts having been dissolved from about them. In older punctures starch could not be detected in the remains of the cell contents; presumably it was hydrolyzed and removed with the rest of the contents, but the process could not be followed in the material available. Herford (14), by allowing *Empoasca solana* De Long to feed upon starch agar, found that a diastatic enzyme hydrolyzing starch to reducing sugars was injected into the punctures. Yeast colonies also developed in the feeding areas on the agar, and as similar yeasts were isolated from the intestine he assumed that they were regurgitated into the punctures and might have been at least partly responsible

PLATE II





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for the starch digestion there. No micro-organisms were detected by the writer in cells injured by *T. pomaria*, but the fixing and staining methods employed may not have been sufficiently delicate to reveal them.

The action of the cytolyzing principle appeared to be confined strictly to those cells into which it was introduced by the mouth parts, no evidence being seen of any diffusion from cell to cell. While no measurements were taken, the area of injury did not appear to enlarge after feeding had ceased, and the uninjured cells adjacent to and scattered among the empty ones usually remained alive, in some leaves for at least four weeks. In older feeding areas some of these isolated cells appeared slightly shrunken or somewhat granular, but the chloroplasts and nuclei were usually normal, the former containing starch grains, so that the shrinkage can probably be attributed to cutting off of their water supply by destruction of the adjoining tissue.

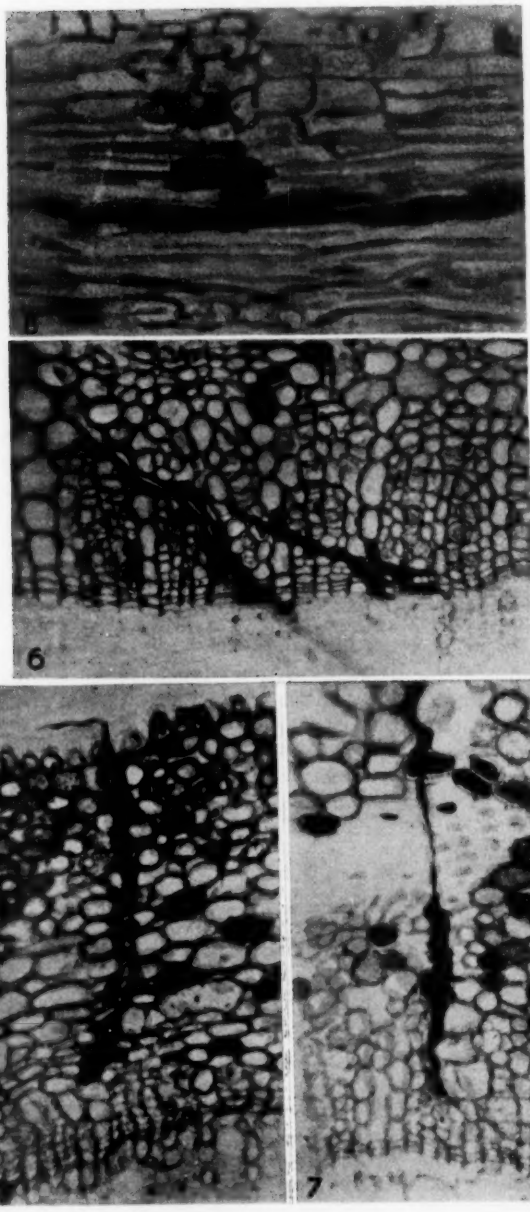
Associated with the puncture through the lower epidermis and extending up through the spongy mesophyll was the sheath material, staining a charateristic bright red with safranin. In the epidermal cell it formed a lining within the puncture or more frequently a solid core, sometimes extending as a short projection on the exterior or as a layer on the cuticle surrounding the mouth of the puncture. Through the spongy mesophyll it partly retained its tubular form or extended as a solid strand of varying diameter through the intercellular spaces, usually ending at or near the lower boundary of the palisade layers, but fine branching strands often extended among the empty cells of the latter. In the spongy parenchyma it was usually attached to some of the cells or occasionally to one of the smaller vascular bundles, but for the most part extended freely through the intercellular spaces. While the strand was usually single, in some cases it branched slightly near the palisade cells.

The staining reactions of the sheath material are so similar to those of the dried residue in the partly emptied cells that it might be supposed the former was derived in some way from the broken down cell contents. That this was not the case was shown in sections cut immediately after feeding, in which the stylet sheath was found to be completely formed, clear and colourless in fresh material and staining the usual deep red in fixed leaves, while the cytolyzed cell contents were very pale green or in stained sections pale red. The finer strands through the empty cells, however, seem to be at least partly formed from cellular materials, as they were apparent as pale greenish strands in fresh sections. Two of the tests which F. F. Smith found to give diagnostic colour reactions with the sheath material of *Empoasca fabae*, namely Millon's and the Hopkins-Cole reactions, gave negative results with *T. pomaria*. This does not necessarily indicate any difference in composition as the same tests also failed when used by the writer on the sheath material of *E. fabae* and *Macropsis* spp.; possibly traces of the preservative interfered with the reactions. From the manner of its formation the sheath material of *T. pomaria* must be of insect origin and is apparently formed from the coagulated saliva.

Adults and all five nymphal instars produced essentially the same type of injury, but in the younger instars there appeared to be a somewhat greater tendency for the punctures to be located near a small vein.

Some nymphs and adults of *T. pomaria* were allowed to feed on drops of agar containing four per cent sucrose placed on glass slides and inverted on the stage of a binocular microscope. The mandibular stylets were observed to be inserted only a short distance, while the very mobile maxillae were forced much deeper and radially in all directions from the entrance. Although the difference in texture between the agar and a leaf might influence the manner of feeding to some extent, movement of the mouth parts in the manner just described would produce the type of injury found on leaves. An attempt was made to fix and section some hoppers with their mouth parts embedded in the leaf, but only a few were successfully sectioned. In these examples the maxillary

PLATE III



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stylets had been withdrawn but the mandibles were still fixed in the epidermis by their barbed tips. Usually the walls of the injured palisade cells are only slightly torn; as the barbed mandibles would be expected to cause much greater laceration of the cells, they are apparently inserted only a short distance below the epidermis. The finer smooth maxillae are then extended more or less obliquely in various directions and used to pierce and remove the contents of the palisade cells. A tendency to force the maxillae obliquely rather than vertically frequently leaves uninjured a group of cells directly above the epidermal puncture, these cells forming the central green spot in the feeding area.

Empoasca fabae (Harris)

The feeding habits of *Empoasca fabae* are somewhat anomalous; although belonging to the Typhlocybinae this species is a phloem feeder and so differs from its near allies, including most species of its own genus, which feed typically in the mesophyll. The various secondary effects which result from the feeding of this species have already been mentioned; De Long (5) has given a brief outline of them which vary greatly on different hosts.

The cause of these symptoms has been in considerable dispute. According to Fenton and Hartzell (9), Eyer (7), and Granovsky (11) they result from the injection of a toxin into the plant while the insect is feeding. Smith and Poos (24) and Johnson (17) concluded that the symptoms were produced by interference with translocation of food materials and water resulting from destruction of the phloem and plugging of xylem vessels. Their cause was not considered in the present studies as it was felt that this aspect of the feeding habits should be approached from the physiological side.

Feeding, or at least visible injury, on apple is confined entirely to young expanding leaves, generally on rapidly growing shoots, and so is of greatest importance on nursery stock. The leaves are curled or rolled downward and inward at the tip and margins, and the areas between the veins often forced up as a result of the retarded growth of the latter to give a somewhat crumpled appearance. Chlorosis is often evident at the tip and margins, and frequently extends inward as somewhat triangular areas along the midvein at the tip and along the proximal side of lateral veins. When extensive feeding is taking place, especially in hot dry weather, wilting of the tip and adjacent margins may occur, sometimes followed by the death and drying up of these parts.

It was found that the feeding habits of this species on apple foliage did not differ in any essential respect from those on alfalfa studied by Smith and Poos; hence they will be considered very briefly. The majority of the punctures were in the midrib and larger veins, but many were also located in the smaller veins and a few led to the smallest vascular strands running through the mesophyll. In the thick-walled collenchyma about the outer part of the larger veins the stylet tracks were largely intracellular, but on entering the deeper parenchyma of looser texture they usually followed a more intercellular course and were often difficult to trace. In the phloem the tracts usually ramified through a considerable area, destroying the tissue and filling it with a dense deposit of sheath material. The xylem was frequently penetrated, usually resulting in plugging of the vessels, but injury in this tissue was much less extensive than in the phloem. The punctures to the small vascular bundles in the blade entered beneath or sometimes at a considerable distance to one side of the bundle, often showing one or two exploratory branches apparently made before the vascular tissue was located. Occasional punctures were found in parts of the blade remote from the veins, usually passing directly upward through the palisade layers and often through the upper epidermis, but there was little or no evidence of feeding in such punctures and none of the extensive probing characteristic of *Typhlocyba* injury.

Macropsis trimaculata (Fitch) and **Macropsis insignis** (Van Duzee)

Although *Macropsis trimaculata* is rather widely distributed in eastern North America it was not considered to be of any economic importance until 1933, when Kunkel (19) found it to be the vector of the virus of peach yellows. His work has been confirmed by Hartzell (12) and Manns and Manns (21). The latter authors also reported the transmission of another virus, little peach, by this insect. An account of the life history and biology of the species in New York has been published by Hartzell (13), and in Ontario by Armstrong and Putman (1).

Macropsis trimaculata, apparently never a very abundant species, was unusually scarce during 1937 and 1938; the small number available was a serious handicap in preparing material for study of the feeding habits. A closely related species, *Macropsis insignis* (Van Duzee), which had been so scare that it was overlooked in previous years, appeared in 1938 in considerable numbers at a few localities and as a scattered infestation over a large part of the Niagara Peninsula. As this species is very similar to *M. trimaculata* in habits, life history, and host plants, it was thought that *insignis* might also be a vector of peach viruses, and experiments were started at Vineland Station to investigate this possibility. For this reason, and to supplement the meagre stock of *trimaculata*, *M. insignis* was included in the present investigations.

Feeding by both species is confined to the twigs and leaf petioles, older slower growing parts of the tree being preferred, particularly the short lateral spurs. The first instar nymphs, hatching soon after the beginning of growth in the spring, gather about the tips of young twigs and especially about the petioles of expanding leaves, but the later instars and adults, although feeding chiefly on growh of the current year, prefer the lower portion of such twigs and are rarely found near the growing tips. They often rest upon one-year-old and older wood but evidently feed upon it to a very limited extent, as it was not possible to maintain adults alive on year-old twigs for more than four days. The following account refers to twigs of the current year's growth unless otherwise stated.

The punctures of both species of *Macropsis* appeared to be of the same nature; there were also no differences in the feeding habits on peach and plum, the two hosts studied. The punctures passed intracellularly through the cortex to the phloem and frequently to the cambium, or more rarely to the xylem. In the epidermis the stylet track passed either through an epidermal cell or intercellularly along the thick lateral walls, in which case the furrows between the convex outer walls probably directed the intercellular passage of the stylets more than any effort on the part of the insect. After withdrawal of the stylets the external opening was usually partly closed by the elasticity of the cuticle, leaving only a narrow passage from 1 to 5 microns in diameter. The stylet track through the cortex was direct and largely intracellular, although in the looser inner portion it sometimes passed partly between the cells. As the stylet fascicle is nearly as large as many of the cortical cells, the contents of the latter were more or less completely destroyed. Some cells adjacent to the puncture, not actually pierced but possibly injured by pressure, showed signs of degeneration, staining more deeply and with granular contents, while others remained apparently normal. The cell walls were very little torn and after withdrawal of the stylets regained their normal shape and size, leaving a passage of varying diameter, usually about 2 to 10 microns. The sheath material formed a well-defined tubular lining, usually extending into the empty cell cavities to give a somewhat moniliform appearance to the sheath.

The thick-walled fibres in the heavy bundles of the pericycle were apparently penetrated by a splitting action, as the course was somewhat angular

and the passage often nearly closed except when plugged by sheath material.

Through the phloem the tubular nature of the puncture was often not apparent and the course less direct. Frequently the track divided into two or more short branches, especially if the cambium was encountered. As the phloem elements were very small the termination of the puncture usually involved several cells, except when a phloem ray was followed inward, when only the single radial row of ray cells might be destroyed. A considerable number of punctures were observed which extended a short distance into the young unlignified xylem, but this appeared to be a more or less accidental occurrence when the insect was feeding in the cambium region. Injured cells in the phloem or cambium were filled more or less completely with a dense deposit of sheath material. Of particular interest from the viewpoint of virus transmission was the penetration of the sieve tubes. As the latter were frequently smaller in diameter than the stylets it was seldom possible to determine the particular cells in which feeding took place, but any puncture penetrating some distance into the phloem would of necessity encounter several tubes except when penetration occurred along a phloem ray. In a few radial sections of injured phloem the sheath material could be seen filling some of the sieve tubes for a short distance above and below the punctures, and the cell contents appeared to be killed or injured for some distance beyond this deposit.

The relative numbers of punctures ending in the phloem or passing through to the cambium varied considerably in different twigs. In sections of 7 seedling plum twigs, each containing 9 to 26 punctures, the proportion reaching the cambium ranged from 12 to 67 per cent. It did not appear to be correlated with either the insect species or the host plant concerned; the physiological condition of the twigs may have been involved but this could not be determined from the small amount of material available. The comparative thinness of the cortex and phloem on the seedling trees from which most of the twigs were taken may have caused an abnormally large proportion of the punctures to reach the cambium, but a few also reached this tissue in normal peach twigs from orchard trees. Of over 200 punctures examined the deepest was 0.51 mm.; it is unlikely that this figure is the maximum, but the latter could not exceed the greatest extension of the maxillae beyond the head capsule, which appeared to lie somewhere between 0.65 and 0.75 mm.

In some twigs a marked preference was shown for the phloem rays. These formed only about 20 to 30 per cent of the phloem in the twigs examined, yet in a few of the latter over half of the punctures were confined to the rays.

It was previously stated that neither species of *Macropsis* can survive for any length of time on twigs of the previous season's growth. In sections of a year-old twig on which an *insignis* adult had fed for two days a couple of normal punctures were found leading into the phloem and a much larger number of short incomplete punctures penetrating the epidermis and periderm or a short distance into the cortex. A somewhat similar condition was noticed on more mature twigs of the current year. The reason why so many punctures were not completed was not obvious, unless the development of a periderm was responsible.

Feeding punctures in petioles were similar to those in stems, but frequently penetrated the xylem for some distance after traversing the phloem.

Comparatively little tissue reaction was noticed in the punctures examined. In a few of the oldest, which had been made more than 20 days before fixation, division had occurred in some cells adjacent to the stylet track. The greatest multiplication had taken place in the region of the pericycle but a few cortical and phloem ray cells had also divided.

The feeding habits of all five nymphal instars of *M. insignis* did not

differ from those of adults except in the smaller size of the punctures. A few of the punctures of the early instars, and a larger proportion of the later, penetrated to the cambium. Nymphs of *trimaculata* were not studied but there was no reason to believe that their feeding habits differed from those of *insignis*.

The presence of the sheath material has already been noted. It was distributed profusely throughout the length of the stylet track and was presumably of the same origin as that of *Empoasca fabae* and *Typhlocyba pomaria*.

The number of punctures made by one insect in a given time is not known, but apparently several may be made in a day. One piece of stem comprising about half the area fed upon for 8 days by an adult *trimaculata* contained 26 punctures and in another twig the same proportion of the area fed upon for 13 days contained 23 punctures. The succulence or other physiological condition of the twigs as well as weather conditions might be expected to influence the number.

DISCUSSION

Since the majority of those Homoptera whose feeding habits were mentioned in the review of the literature are phloem feeders, this habit may be accepted as typical of the order as a whole. Phloem feeding is therefore apparently the primitive condition among the Cicadellidae, as exemplified by *Macropsis trimaculata* and *M. insignis* of the subfamily Bythoscopinae; *Homalodisca triquetra, Graphocephala versuta* and *Draeculacephala mollipes* (Cicadellinae); and *Eutettix tenellus* and *Cicadulina mbila* (Jassinae). In the subfamily Typhlocybinae alone as represented by *Typhlocyba pomaria,* most species of *Empoasca* and others a different method of feeding has been developed, the majority, at least of American species, attacking the mesophyll of the foliage. The Typhlocybinae is the most highly specialized division of the Cicadellidae, as shown by reduction of wing venation and usually loss of ocelli, and morphological specialization has apparently been paralleled by evolution of distinctive feeding habits. *Empoasca fabae,* feeding in phloem, may be regarded as an otherwise typical member of this subfamily which has either retained or reverted to more primitive habits.

The very specific relationship existing between certain plant viruses and their respective leafhopper vectors has suggested the possibility of some peculiarity in the feeding habits of these species which especially fit them for virus transmission. Among the Hemiptera in general K. M. Smith concluded that there was little or no relation between feeding habits and virus transmission, except possibly in such forms as mirids where the very toxic saliva might interfere with successful inoculation. Within the Cicadellidae, however, differences are found which, while possibly not excluding transmission, would at least affect its efficiency. It is significant that no fully authenticated cases are known of the Typhlocybinae being involved in virus transmission, although the group is one of the largest in numbers of both species and individuals. The three vector leafhoppers whose feeding habits are known, namely *Cicadulina mbila, Eutettix tenellus,* and *Macropsis trimaculata,* are all phloem feeders. Furthermore, Storey (27) found that maize could be infected with the virus of streak disease only when the stylets of *Cicadulina mbila* entered the phloem, and Fife and Frampton (10) obtained much more efficient transmission of the virus of curly top when *Eutettix tenellus* was able to penetrate to the phloem of beet. The mesophyll-feeding Typhlocybinae, which rarely penerate vascular tissue, would therefore be relatively inefficient vectors of viruses of this type. The rapidity with which the cells are killed and their contents extracted would also militate against successful transmission, as the cell into which a virus is introduced must apparently remain alive for some time to cause infection of the plant. In brief, the feeding habits of virus-transmitting leafhoppers do not

exhibit any special features but are of the generalized phloem-penetrating type common to the majority of Homoptera, while species of more specialized mesophyll-feeding habits are not known to act as virus vectors.

If we assume that the sheath material is formed of salivary secretion and that the latter is the specific carrier of infective virus, the deposition of this material in all tissues of the twig except the pith would ensure inoculation of the tree by *Macropsis trimaculata* even if introduction of the virus into the phloem was not specifically necessary. Storey (27) emphasizes that it is difficult to accept the instantly coagulating and highly insoluble sheath-forming saliva of leafhoppers as the viruliferous substance, although he can offer no alternative hypothesis. In *Typhlocyba pomaria*, the saliva forming the sheath deposited along the lower part of the stylet track must be quite different from that causing cytolysis of the palisade cells but leaving no deposit within them. Furthermore, Dobroscky (6) found that the salivary glands of *Macrosteles divisus* (Uhler) (=*Cicadula sexnotata* American authors, not Fallen) were composed of five types of cells showing cytological differences and presumably secreting different substances. It is realized, however, that the observations on *Typhlocyba* should be applied with caution to species such as *Macropsis* and *Cicadulina* which have quite different feeding habits. Storey (27) was unable to detect the emission of any material other than that forming the sheath from the mouth parts of *Cicadulina mbila*.

Summary

The cicadellid *Typhlocyba pomaria* McAtee, which produces white mottling of foliage typical of members of the subfamily Typhlocybinae, feeds in the palisade layers of the mesophyll of apple leaves. The cells are punctured and their contents removed after cytolysis by the saliva, which appears to act only on the cells into which it is injected and does not diffuse through the tissues. Vascular tissue appears to be only accidentally penetrated. A stylet sheath, apparently of salivary origin, is deposited along the outer part of the stylet track.

The feeding habits of *Empoasca fabae* Harris on apple are similar to those on other hosts reported by Smith and Poos, feeding being confined chiefly to the phloem, although xylem and other tissues are occasionally penetrated.

Macropsis trimaculata (Fitch), the vector of peach yellows and little peach viruses, feeds primarily in the phloem of peach and plum twigs and petioles. Considerable feeding is also done in the cambium and the xylem is occasionally penetrated. The path of the stylets is chiefly intracellular and lined with a well-defined stylet sheath. The feeding habits of *Macropsis insignis* (Van Duzee) are identical with those of *M. trimaculata*.

It is concluded that the feeding habits of virus-transmitting leafhoppers do not show any special features but are of the generalized phloem-penetrating type which is apparently the primitive condition in the Cicadellidae and among Homoptera in general. Members of the subfamily Typhlocybinae, in which the more specialized habit of feeding in the mesophyll has been evolved, do not appear to be concerned with virus transmission.

ACKNOWLEDGMENTS

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EXPLANATION OF PLATES II AND III.

Fig. 1. Feeding areas of *Typhlocyba pomaria* on apple leaf. X 4.6.

Fig. 2. Section through a feeding area of *Typhlocyba pomaria*, showing deeply stained sheath material and empty palisade cells. X 285.

Fig. 3. A stylet sheath of *Typhlocyba pomaria*, somewhat thicker than usual. X 285.

Fig. 4. Section of a feeding area of *Typhlocyba pomaria* fixed three or more weeks after feeding, showing isolated living cells among empty ones. X 285.

Fig. 5. Section through a puncture of *Macropsis insignis* extending to outer phloem of plum twig. X 285.

Fig. 6. Section through a branched puncture of *Macropsis trimaculata* ending in cambium of plum twig. X 285.

Fig. 7. Section showing enlargement and division of phloem ray cells adjacent to a puncture of *Macropsis trimaculata* in plum twig. X 285.

Fig. 8. Radial section through phloem of plum twig showing terminus of a puncture of *Macropsis insignis* (penetrating from above). Injury to seive tubes is evidenced by deeply staining contents. X 285.

NOTES

NOTE ON THE SPECIES OF *AGABINUS* (COLEOPTERA, DYTISCIDAE)

During a recent hurried auto trip from Vancouver, B.C., to Vernon, short stops were made at various small streams and ponds. Most of the aquatic beetles found are common species, but two of the records are extremely interesting.

Agabinus sculpturellus Zimm., 1919. Zimmermann described this species from a single female from Shasta Retreat in northern California; it is still rare in collections. I have a male from Carrville, Trinity Co., Calif. (R. M. & G. E. Bohart), and a female from Myers Creek, near Pistol River, on the coast of Oregon (H. Leech). Two males were found clinging to debris in a tiny roadside stream just north of Alexandra Lodge, about thirty-two miles north of Hope, B.C. This is a northward extension of at least 500 miles in the reported range of the species.

Agabinus glabrellus (Motschulsky), 1859. Described from California, where it is common in mountain streams; Zimmermann (1919:205) has recorded it from New Mexico, and I have seen a Texas specimen in Mr. J. B. Wallis' collection. A single female was found in a pool of water at the end of a small irrigation flume, about a mile and a half west of Ashcroft Manor, near Ashcroft, B. C. This extends the known range of this species by several hundred miles, since Hatch (1939) records it from eastern Washington, in his mimeographed "Preliminary List of The Coleoptera of Washington".

The two known species of *Agabinus* may be separated by the following key:

Ovate; elytra finely punctate, punctures more conspicuous than the lightly engraved reticulation; lines of reticulation fine, meshes (enclosed areas) irregular in size and shape, not at all concave; metasternal wings broad, shortest distance between mesocoxa and metacoxal plate three-fourths width of latter, measured along same line ..... *glabrellus*

Broadly oval; elytra very finely punctate, punctures less conspicuous than the reticulation; meshes irregular in size and shape, smaller and more numerous than in *glabrellus*, most of them concave, giving elytra a dimpled appearance; metasternal wings narrow, shortest distance between mesocoxa and metacoxal plate less than two-fifths width of latter, measured along same line .... *sculpturellus*

Hugh B. Leech,
Vernon, B.C.

THE ATTENDANCE OF EXTRAFLORAL NECTARIES OF *SAMBUCUS RACEMOSA* L. BY THE ANT, *LASIUS NIGER* VAR. *NEONIGER* EM.

Myrmecological literature is not lacking in references to the attendance of the extrafloral nectaries of a number of vascular plant species by ants. The following is a note on a specific relationship which was observed from mid-July until the end of August, 1938, at Ash Point, Maine.

Workers of *L. neoniger* were observed to file up and down several *S. racemosa* bushes. When a casual glance did not reveal the presence of aphids, closer observation showed that the stipules of the younger branches gave off saccharine droplets which the ants were imbibing. In feeding, the workers always stroked the sides of the stipules much in the fashion that aphids are caressed. The returning files of workers had well distended gasters, in striking contrast to those ascending. They attended the stipules assiduously from early morning until late into the evening.

Examination of many of the numerous *S. racemosa* bushes in the region showed *L. neoniger* workers to be present and attending the stipules in almost every case.

During the course of the summer two other ant species were observed at stipules. Workers of *Formica fusca L.* var. were observed feeding in several cases and when they met a *L. neoniger* worker on or near the bushes where feeding took place, they always fled posthaste. The stipule feeding activities of the *Formicae* were, therefore, rather sporadic due possibly to this hampering influence. On one occasion a single worker of *Tapinoma sessile* (Say) was seen feeding at a stipule.

Merle W. Wing,
University of Minnesota.

LEAD OR TIN TUBES IN A BIOLOGICAL LABORATORY

For some years now we have discarded glass dropping bottles for Canada balsam in favor of what are described in trade as "Ophthalmic tin tubes with noses". These are of lead, like tooth-paste tubes, but have half or one inch-long screw-top snouts and are obtainable from wholesale firms supplying corks and other such items to drug stores. We use two sizes 3½ x ½ inch and 4 x 1 inch, which cost locally from six to twelve dollars per gross at time of writing (September, 1940); the price has practically doubled in the last year owing to war conditions. The tubes come with the butt ends open and, with a little practice, are readily filled with diluted Canada balsam, cedar oil, liquid glue, paste, petrolatum or any other laboratory material of a like consistency, and the bases are then folded over twice and crimped. Ordinary office paste bought in large bottles should be liquified in a hot water bath to facilitate pouring. The tubes are then issued to students in place of dropping bottles and are infinitely cleaner and handier than any other receptacles we have used. A 3½ x ½ inch tube holds enough balsam for making about two hundred slides using 12 mm., round coverslips.

The only drawback in connection with these tubes is that they cannot be used for any medium containing acetic acid, such as Berlese fluid, egg albumen or our modification of De Faure's fluid to which we add a few drops of acetic acid, as the lead breaks down in the course of two or three months.

We got the idea from Mr. Charles Anders, druggist of Vancouver, who supplied us some eight years ago with prepared Canada balsam put up in this fashion.

G. J. Spencer,
The University of British Columbia.

A NOTE ON OVIPOSITION BY THE QUEEN BUMBLEBEE, *BOMBUS AMERICANORUM* FABR.

Bombus americanorum is one of the many species of bumblebees on which little or nothing is known of the egg-laying habits of the queen. Frison (Ann. Ent. Soc. America, 23.645, 648, 1930) found eggs of this species in small waxen cavities made by the queen, each cell containing from three to five eggs, and I have also recorded for this species (Bull. Brooklyn Ent. Soc. 32:6, 1937) the same method of depositing eggs in queen-made cells of pollen-paste. Sladen, evidently basing his remarks on observations on *Bombus lapidarius*, give in his book on *The Bumblebee* (p. 9, 1912), a detailed account of this aspect of queen behaviour. He states that the queen, with her jaws, works the top of a lump of pollen-paste into a circular wall and into the cell so formed drops her first batch of eggs, sealing it over with wax as soon as they have been deposited. The young larvae feed on the pollen-paste of their bed, and this paste is replenished by the queen from time to time as it is consumed. She also feeds to them occasionally a regurgitated mixture of pollen and honey which she injects into the cell through an aperture made by her jaws. Sladen shows figures of several cells, each with from six to eight young larvae living together in apparent contentment.

A recent observation by me on queen *B. americanorum* shows a method of oviposition quite different from the aforementioned behaviour. The queen in question, whose nest I discovered in an abandoned house near Marthasville, Missouri, on May 30, 1940, did not deposit her eggs in a spacious cell but imbedded them singly in a small mass of pollen-paste. The nest, located in a pocket near the top of a straw pile, consisted of only a waxen honey-pot, three-fourths full of honey, with a roll of pollen-paste attached to the bottom containing the eggs. This egg-bearing mass was roughly oval: one-half inch high, three-eights inch wide, and five-sixteenths inch thick. It contained eight eggs and one small larva. The eggs, each individually placed, were so tightly packed in the surrounding medium that a dissecting needle was employed to remove them, while the one larva, having already eaten away sufficiently of the surrounding wall, had plenty of room.

With the feeding of the larvae in their individual positions, the barriers of wall gradually break down, and the hitherto solitary young eventually find themselves thrown together in one cell in a state of socialization which would make it easily possible for the queen to resort to mass feeding in the way described by Sladen for another species.

B. americanorum evidently resorts to two methods in egg-laying, that of imbedding the eggs individually in the pollen-paste as well as that of dropping several of them in a roomy cell. It is interesting to know that *B. americanorum* is not the only bumblebee that resorts to these two methods of oviposition, for Sladen found another species, *Bremus ruderatus* (loc. cit. p. 273), also employing the same two methods. He found fourteen eggs in one nest, each occupying a separate bed in the pollen, and in two other nests of the same species he found three eggs in one cell and five in another.

Incidentally, it is of interest to note that the small size of this colony on so late a date as May 30 substantiates Frison's statement (loc. cit. 23:658, 1930) that the queens of *B. americanorum* commence their nesting activities very late in the spring.

Phil Rau, Kirkwood, Missouri.

THE VERY EARLY STAGES OF NEST-FOUNDING BY *POLISTES PALLIPES*

Because a mother *pallipes* chose to build her nest outside my study window, I am able to record the very first movements of her nest-founding behaviour. I first saw her on May 7 as she entered a small crack in the window screen, her jaw filled with wet paper pulp, and moved rapidly half way up the frame to a small stem of pulp, the foundation of her future nest. To this stem she added her load, thereby increasing its length and its thickness. Two more trips for pulp, which consumed fifteen or twenty minutes respectively, gave her sufficient material to fashion her first shallow cup at the bottom of the stem. Into this cup she placed her egg, attaching it to its ceiling, but because of its shallowness, half of the egg was exposed to view. Two additional trips gave her enough pulp to enlarge the cell sufficiently to conceal the egg. A downpour of rain lasting several hours, while she was foraging, prevented her from returning that afternoon, and she spent the night away from the nest. The next morning she was again on the nest and was seen on it thereafter every day until the time of her disappearance on June 17.

During the first ten days after the stem and cell were made, much time was spent on the nest with little or no work done; this was probably due to the several cold days which followed the rain (the temperature often dropping during this period to 54 degrees F.,) and not to the laziness of the queen. With the rise in temperature on May 17 and the continued warmth for several days, the queen added six cells to the nest (one-third full size) and did this in two days, on May 18 and 19. Each of the seven cells now contained an egg, and the queen spent the next six days adding layer upon layer of pulp to the cells, bringing in food for the one larva in the first cell constructed, and probably also to the others which may by that time have hatched from the later eggs, and also in gathering stores of honey to place with the young as well as in foraging for her own food.

On June 17, forty days after commencing the nest, the queen went on a foraging trip and never returned. Shortly before that I placed a white mark on her thorax so that I would be able to distinguish her from the workers which I expected to appear soon on the nest. The color might have made her a mark for enemies, and she probably fell prey to some hungry bird or beast. At any rate, an examination of the nest, several days later, proved that there was but little idleness in her short life, for in that short space of time her industry had produced a nest of fourteen cells, one of which contained a pupa, five larvae of various sizes, four contained eggs, and four were newly made empty cells.

Thus ended Act 1 of a Missouri *pallipes* colony. Act 2 will begin when the first worker leaves the cell and assumes, in a queen-like fashion, the care and responsibility of the orphaned brood.*

Phil Rau, Kirkwood, Missouri.

*For details of the behaviour of Act 2, see Turner's "An Orphan Colony of *P. pallipes*", Psyche 19:184-190, 1912; and Rau "Orphan Colony of Polistes", Ent. News 40:226-232, 257-259, 1929.

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